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Actinomycetes are one of the most important sources of pharmacological and industrial relevant natural products (Weber, T., et al, 2015). Unfortunately, many of the wild-type strains are recalcitrant to efficient genetic manipulation approaches, which is a severe bottleneck for systematic metabolic engineering. We developed a genome editing toolkit based on CRISPR-Cas9, which includes three sub-systems, pCRISPR-Cas9, pCRISPR-Cas9-ScaligD, and pCRISPR-dCas9 (CRISPRi), for genome editing and modulating transcription of target genes (Tong, Y., et al, 2015; Tong, Y., et al, 2016). The system is widely used in our section and the actinomycete community. Although the toolkit significantly speeds up genetic engineering for many strains, there are still limitations: for instance, often non-model actinomycetes cannot be transformed using the standard protocols, are resistant to the standard antibiotics used for plasmid selection, or cannot replicate commonly used plasmids; there is no very good spacer finder for non-model organisms; the current system cannot meet the high throughput and automation genome editing purpose. In order to address these limitations, we extended our actinomycete CRISPR-Cas9 toolkit: a spacer finder, CRISPy-web (Blin, K., et al, 2016) was released to facilitate the sgRNA design for non-model organisms, which can design sgRNAs for any microbial genome. A prototype of a "DNA-free" genome editing system for actinomycetes was demonstrated by inactivating actinorhodin production in the model strain *S. coelicolor*. An USER-based multiple sgRNA assembly strategy (Tong, Y., et al, 2017) was developed and validated for automated high-throughput cloning. This update of our CRISPR-Cas9 toolkit will further extended its applicability for actinomycetal genome editing.

*References:*

[1] Weber, T., Charusanti, P., Musiol-Kroll, E., Jiang, X., Tong, Y., Kim, H.U., Lee, S.Y., (2015) Metabolic engineering of antibiotic factories: new tools for antibiotic production in actinomycetes. *Trends Biotechnol.* 2015, 33, 15-26.

[2] Tong, Y., Charusanti, P., Zhang, L., Weber, T., Lee, S.Y., (2015) CRISPR-Cas9 Based Engineering of Actinomycetal Genomes. *ACS Synth. Biol*. 14(9):1020-9.

[3] Tong, Y., Weber, T., Lee, S.Y., CRISPR/Cas9-based system. 2015, EU Patent. EP15160126.7.

[4] Blin, K., Pedersen, L., Weber, T., Lee, S.Y., (2016) CRISPy-web: An online resource to design sgRNAs for CRISPR applications. *Synthetic and Systems Biotechnology*. DOI:10.1016/j.synbio.2016.01.003

[5] Tong, Y., Robertsen, H.L., Blin, K., Weber, T., Lee, S.Y., "CRISPR-Cas9 toolkit for actinomycete genome editing." in *Synthetic Metabolic Pathways.* Eds. Jensen, M.K., Keasling, Jay D. New York: Humana Press, 2017, in press.

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